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Trait Anxiety and Salivary Cortisol During Free Living and Military Stress

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Introduction: Accumulating evidence suggests that negative affect is associated with elevated cortisol. Limited research has investigated this association in young, highly functioning, and stress-resilient populations. **Methods:** We examined the relation of trait anxiety with total and diurnal salivary cortisol during free-living conditions and during a stressful military exercise in 26 military men ages 19–30 yr ($M = 21.6$, $SD = 2.3$). Salivary cortisol was assessed at five time points over 2 consecutive days of free-living measurement, and three time points during a stressful military experience. Trait anxiety was measured with the trait portion of the Spielberger State-Trait Anxiety Inventory 1–3 wk prior to the military exercise. **Results:** Total cortisol concentrations were similar between men reporting high or low anxiety during free-living conditions (8.6 ± 3.2 vs. 7.4 ± 2.8 nmol \cdot L⁻¹, respectively, $P > 0.05$), and military stress (21.3 ± 7.3 vs. 19.0 ± 7.0 nmol \cdot L⁻¹, respectively, $P > 0.05$). The diurnal cortisol profile differed significantly ($P = 0.04$) between these men during the free-living condition, but not the stressful military experience ($P > 0.05$). Specifically, during free living, men with low anxiety exhibited a diurnal cortisol pattern that peaked in the early morning, decreased precipitously during the midmorning, and continued to decrease throughout the day, reaching a nadir in the evening. By contrast, the cortisol pattern of high-anxiety men remained elevated and significantly higher than their low-anxiety counterparts during the midmorning, decreased more slowly throughout the day, and reached its lowest level in the evening. Results were not substantially altered following adjustment for sleep duration or wake time. **Conclusion:** These findings suggest that trait anxiety influences the diurnal cortisol pattern in young, apparently healthy men during free-living conditions, but does not predict the cortisol response to uncontrollable military stress.

Keywords: stress, hormones, diurnal patterns.

STRESS IS BROADLY defined as an imbalance in physiological systems that activates both physiological and behavioral responses to restore balance (6). The physiologic stress response, in turn, is generally believed to be regulated by at least two closely related systems—the hypothalamic-pituitary-adrenal (HPA) system, and the sympathetic-adrenal-medullary system. The sympathetic-adrenal-medullary system involves preganglionic sympathetic fibers that directly innervate the adrenal medulla, prompting the secretion of adrenaline (epinephrine) and noradrenaline (norepinephrine). The HPA system is a complex set of interactions between the hypothalamus, the pituitary gland, and the adrenal glands. Upon detection of threat, emotional responses

are generated in cortical and limbic (e.g., amygdalar and hippocampal) brain structures, which, in turn, triggers the activity of the hypothalamus. The hypothalamus produces corticotropin-releasing hormone, which then activates the anterior pituitary gland to produce adrenocorticotrophic hormone. Adrenocorticotrophic hormone, in turn, stimulates the adrenal cortex to produce and release glucocorticoid hormones (6).

The primary glucocorticoid is cortisol, which affects many body tissues, including the brain. Cortisol mobilizes energy for action (i.e., fight or flight) and suppresses less immediate physiological processes (such as immune function) during stress (14,20). It is well established that cortisol has a strong diurnal cycle characterized by high levels in the morning followed by a continuous drop throughout the day (12,29). Accumulating evidence, though, suggests possible moderating effects of psychosocial and environmental factors on diurnal cortisol patterns in both healthy and clinical populations. Current research suggests, for instance, that flattened diurnal cortisol patterns are associated with repressiveness and anxiety (10,32), less positive affect (22), hours of maternal employment and number of children in the household (3), material hardship (24), and depression (8,32). Furthermore, flattened cortisol rhythmicity has not only differentiated breast cancer patients from healthy controls (1), but has also predicted fatigue (4) and shorter survival among women with breast cancer (27). By contrast, positive affect (22) and religiosity (7) have been as-

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sociated with diurnal cortisol profiles characterized by higher morning values and lower evening values. Interestingly, higher morning peaks and lower evening nadirs in salivary cortisol have also been observed in unemployed subjects in comparison to an employed group, suggesting that employed subjects may experience greater allostatic load (21). Taken together, this set of findings broadly suggests that greater psychosocial stressors produce a relatively flattened diurnal cortisol rhythm.

In addition to understanding individual differences in daily cortisol rhythmicity, it is also important to understand individual differences in responses to stressful experiences. Over the years, a body of research has documented physiologic stress responses to diverse military environments including naval aviation (26), basic combat training (25), dive training, special operations (23), and other acute training-related scenarios (31). In turn, a line of research investigating individual differences in responses to military survival training has begun to explore this area. Morgan and colleagues, for instance, have demonstrated robust increases in cortisol concentrations in response to Survival, Evasion, Resistance, and Escape training (SERE)—a harsh and realistic environment in which students are taught to survive, evade enemy captors, resist exploitation, and escape during captivity (17–19)—and have further explored individual differences by way of elite vs. less elite warfighter comparisons. In one such study (19), no significant differences were observed between elite Special Forces and non-Special Forces Army soldiers from baseline to acute stress with respect to cortisol, although Special Forces soldiers exhibited greater responses in plasma norepinephrine. In other work, these scientists have demonstrated greater neuropeptide Y responses to survival training in elite warfighters compared to their less-elite counterparts (17,18).

Little or no research has examined the relation of psychosocial characteristics to diurnal cortisol rhythmicity in high functioning, stress-resilient populations. Therefore, it is unknown if psychosocial variables may influence daily cortisol patterns in such a population. Confirmation in this population would strengthen our understanding of the biological responses to daily psychosocial stressors. Additionally, there is little existing literature documenting the influence of psychosocial characteristics on physiologic responses to realistic military stress. A purpose of the present study was to determine the influence of trait anxiety on the total cortisol concentration and diurnal cortisol pattern during free-living conditions in a young, stress-resilient sample of military men. In turn, a second purpose was to examine the influence of trait anxiety on cortisol responses to a stressful military exercise. It was hypothesized that high-anxiety participants would exhibit a higher, flatter diurnal pattern in comparison to their low-anxiety counterparts during daily living. In light of the paucity of existing literature, the investigation of trait anxiety and cortisol reactivity to survival training was considered exploratory.

METHODS

Participants

Active duty Navy personnel were recruited as part of a larger study designed to examine the stress responses associated with military survival training. Recruitment of subjects was performed between January 2006 and April 2007 by the principal investigator. Subjects were Navy members who had recently completed Aviation Warfare/Rescue Swimmer or Basic Underwater Demolition/SEAL training. Specifically, each Aviation Warfare member had recently completed technical training and had graduated or were awaiting graduation, after which they were scheduled to attend SERE training. Each Basic Underwater Demolition/SEAL graduate was awaiting SERE training as a component of the 6-mo Seal Qualifying Training, a 6-mo course of advanced training experiences completed prior to receiving the SEAL warfare designation. Potential subjects were excluded from the study if they 1) had ever had a head injury, concussion, or lost consciousness for any period of time; 2) reported having posttraumatic stress disorder; 3) reported substance dependence of any kind; or 4) reported taking prescription or over-the-counter medications of any kind.

Of the 28 men who were recruited, we excluded two who were noncompliant with the free-living salivary cortisol sampling procedures. Thus, the current analyses are based upon a sample of 26 male sailors ages 19 to 30 yr (mean age: 21.6 yr, SD = 2.3; body mass index: 24.0 kg · m⁻², SD = 1.7). The majority of participants were Aviation Warfare/Rescue Swimmer students (76.9%, N = 20) and the rest were students in Navy SEAL Qualifying Training (N = 6). The average number of years of military experience was 1.7 (SD = 0.9). Prior to participation, all subjects provided written informed consent and the study was approved by the Institutional Review Board for human subjects research at the Naval Health Research Center.

During an initial meeting with research staff, participants completed demographic and psychometric questionnaires, and received training for the standardized collection of free-living saliva samples. A standard demographic questionnaire was used to obtain body-weight and height, educational level, duration of military service, and military occupational specialty. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Participants were asked to report the results of their most recent Physical Readiness Test (PRT). Military personnel are required to maintain a standard level of physical fitness by scoring satisfactorily during a PRT administered twice per year. The time required to complete a 1.5-mile run on a standardized course as part of the PRT was used as a measure of aerobic fitness (lower values indicate higher fitness).

Since cortisol concentrations have a known relationship to morning awakening (33), both duration of sleep and morning wake time were measured with the use of the Octagonal Basic Motionlogger Actigraph (Precision

Control Design, Ft. Walton Beach, FL) during the free-living cortisol measurement period (described in detail below). The Motionlogger Actigraph is a wrist-sized accelerometer and microprocessor that measures physical movement, or for the purposes of the present study, the lack of movement. Wake time was standardized as the number of minutes from the 0730 data collection time point. For example, a wake time of 30 or -30 would equate to a wake time of 0800 and 0700, respectively. Self-reported sleep information was substituted for two participants who were missing these data from the Motionlogger Actigraph.

Total and Diurnal Salivary Cortisol

Baseline salivary samples were obtained over the course of 2 consecutive days, 1-3 wk prior to the start of survival training. The saliva samples were self-collected by participants in a free-living setting and collected in Salivette tubes (Sarstedt, Inc., Newton, NC), which were individually labeled with the date and time of sampling. Specifically, the samples were collected during the work-week (either Mon-Tues or Wed-Thur), and all participants participated in typical daily work routines (approximately 0730 to 1630). Participants were instructed not to brush or floss their teeth, eat, or drink, and to rinse their mouth with cool water immediately prior to sampling. They were also asked not to engage in physical activity during the 2-d sampling period. Participants signed a statement of compliance each day during this period stating that they had not used any caffeinated food or beverages, alcohol, tobacco products, or medications 12 h prior to or during the data collection period. On each day, saliva samples were taken at 0730, 0830, 0900, 1600, and 1930. A greater concentration of samples was administered during the morning hours to capture the expected morning surge and subsequent decline during the midmorning hours with greater resolution.

During the captivity portion of SERE training, salivary samples were collected by study staff members on a single day at three time points (0900, 1600, and 1930). Logistical limitations during SERE training dictated that we reduce the frequency of specimen collection. Samples were collected in Salivette tubes (Sarstedt, Inc.), which were individually labeled as to the day and time of sampling.

Samples were kept in a portable cooler during sampling and then, once returned, were immediately stored at -70°C . Salivary cortisol was measured using a radioimmunoassay kit (Coat-A-Count Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the assay was $0.69\text{--}55.2\text{ nmol} \cdot \text{L}^{-1}$ and intra- and interassay coefficients of variation were 3-7% and 5%, respectively.

Of the 260 expected saliva samples during the free-living condition, 24 (9.2%) included either too little saliva to obtain a reliable cortisol measurement or were missing due to unknown reasons. Of the 78 expected samples from the stressful military exercise condition, 11 (14.1%) included too little saliva. Total cortisol levels for each condition were calculated by taking the average

concentration across all collection times, including each of the 2 sampling days for the free-living condition. To display the diurnal variability in cortisol levels, the average concentration at each collection time during the free-living condition was calculated.

Trait Anxiety

Self-report of anxiety was assessed with the trait portion of the Spielberger State-Trait Anxiety Inventory. The trait anxiety inventory includes a series of 20 items, which asks respondents to describe how they generally feel using a 4-point Likert-type scale (almost never, sometimes, often, almost always). Examples of items include, "I feel pleasant," "I worry too much about something that does not matter," and "I make decisions easily." The trait anxiety inventory is scored by reverse coding each positive item and then summing across all items. Scores range from 20 to 80, with lower scores indicating less anxiety and higher scores indicating a greater level of anxiety. The scale is widely used, and its reliability and validity has been established in several different populations (28). Internal reliability of the trait anxiety scale in the current study was acceptable (Cronbach $\alpha = 0.79$). A median split technique was used to identify high and low-anxiety groups.

Military Survival Training

SERE training is described in an earlier report (30). Briefly, United States military members at high risk of capture are required to attend this course, which includes a period of confinement in a Resistance Training Laboratory (RTL). After an initial phase of classroom-based didactic training, students are taken to the field where they receive applied training in survival, evasion, resistance, and escape techniques. Students are then released into the field and tasked with the goal of evading enemy captors. Upon eventual capture, students are taken to the RTL, where they are expected to apply their recently learned skills of resistance to political indoctrination and captivity-related problems. Since SERE training is designed to simulate the prisoner-of-war experience, it offers a unique medium in which to study the effects of highly realistic captivity stress on human functioning. As noted above, the cortisol data were collected during the RTL (captivity) portion of SERE training on a single day at three time points (0900, 1600, and 1930). The details of this captivity portion are classified, which prevents us from explicitly describing scenarios that took place during this phase of training. In general, all participants were systematically exposed to a series of highly stressful captivity-related problems and dilemmas, during which they were required to exhibit several target behaviors and resistance techniques learned during the classroom phase of training.

Statistical Analyses

Continuous and discrete characteristics according to anxiety are displayed as the mean with SD or percent,

respectively. Since cortisol concentrations were positively skewed, all hypothesis tests were based upon log-transformed cortisol; untransformed means are reported for ease of interpretation. Descriptive characteristics according to trait anxiety groups were compared with independent sample *t*-tests for continuous measures and Fisher's exact tests for categorical measures. A repeated measures analysis of variance (ANOVA) was used to evaluate whether the diurnal profile of cortisol differed significantly by anxiety classification. To further explore the influence of wake time on the diurnal cortisol profile during the free-living condition, the repeated measures ANOVA was repeated, adjusting for wake time as a covariate. When a statistically significant overall effect of anxiety on the diurnal cortisol profile was confirmed, independent *t*-tests were used to compare mean cortisol concentrations between high- and low-anxiety participants at each time point. An ANOVA was also used to examine group differences in cortisol reactivity to the stress of SERE training. All hypothesis tests were two-sided and the probability of committing a type I error was set at 0.05. SPSS version 15.0 (SPSS Inc., Chicago, IL) was used to perform all analyses.

RESULTS

Overall, no significant differences were noted between men of high or low trait anxiety with respect to age, height, weight, BMI, years of military service, aerobic fitness, total sleep time, total salivary cortisol during free living, or total salivary cortisol during military stress. Men of high anxiety had a wake time, on average, approximately 45 min later than men of low anxiety ($P = 0.04$). As expected, higher anxiety scores were observed in men classified with high anxiety ($P < 0.0001$).

In Fig. 1, we display the diurnal pattern of salivary cortisol during the free-living condition by presenting mean untransformed cortisol concentrations according to time of collection and trait anxiety. A repeated measures ANOVA confirmed a significant overall difference in the diurnal salivary cortisol profiles between men of

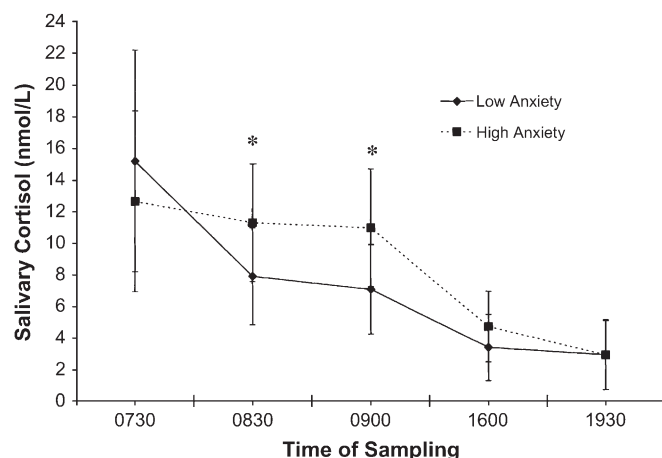


Fig. 1. Mean (SD) diurnal salivary cortisol concentrations according to trait anxiety in military men during free-living conditions. * $P < 0.05$ for mean difference in salivary cortisol at 0830 and 0900.

high and low anxiety ($P = 0.04$ for the interaction of time and anxiety). The inclusion of wake time as a covariate within the repeated measures ANOVA did not substantially alter the observed association of diurnal cortisol with trait anxiety (P for the interaction from 0.04 to 0.09). Low anxiety participants exhibited a diurnal cortisol pattern that peaked in the early morning at 0730, decreased precipitously during midmorning, continued to decrease throughout the day, and reached a nadir in the evening. High anxiety participants, however, displayed a cortisol pattern that remained elevated and significantly greater than the cortisol concentrations of men with low anxiety during the midmorning, and then decreased throughout the day, reaching its lowest level in the evening. The anxiety groups also differed at the 0830 and 0900 time points relative to the 0730 time point (0830 vs. 0730, $P < 0.05$; 0900 vs. 0730, $P < 0.05$), further elucidating the group differences with respect to the midmorning decline in cortisol.

The salivary cortisol profiles during military stress are displayed in Fig. 2. Although cortisol levels were significantly ($P < 0.05$) elevated at each sampling time with respect to free-living conditions for both the low- and high-anxiety men, we found no evidence to suggest that the diurnal profile during military stress differed between these groups ($P > 0.05$).

DISCUSSION

Since accumulating evidence suggests that negative affect is associated with elevated cortisol and limited research has investigated this association in stress-resilient populations, we examined the relation of trait anxiety to total and diurnal salivary cortisol, and to cortisol responses to stressful training in military men. Although total cortisol values did not differ significantly between high- and low-anxiety groups, discrepant diurnal cortisol profiles were observed. Low-anxiety men exhibited an early morning peak in cortisol followed by a more dramatic downward shift midmorning, while the profiles of high-anxiety men remained elevated throughout the midmorning hours. The two groups differed significantly in cortisol levels specifically in the midmorning hours (i.e., 0830 and 0900). With respect to the stress of

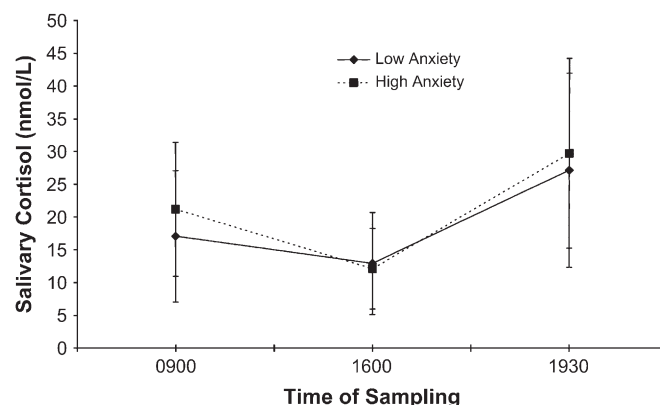


Fig. 2. Mean (SD) diurnal salivary cortisol concentrations according to trait anxiety in military men during military stress.

survival training, both groups demonstrated dramatic cortisol increases in response to SERE, but no group differences prevailed.

In this study, different diurnal cortisol profiles were observed between high- and low-anxiety groups. These findings complement and extend a recent body of evidence suggesting that maladaptive psychosocial functioning and challenging environmental circumstances are linked to flattened diurnal cortisol profiles in a variety of healthy and clinical populations, which, in turn, has been quantified as a possible risk factor for morbidity and early mortality (27). Giese-Davis and colleagues (10), for instance, demonstrated flatter diurnal salivary cortisol slopes in association with repression and high anxiety in women with breast cancer, despite no relationship to overall or morning cortisol values. In another study (1), women with breast cancer have demonstrated significantly flatter diurnal cortisol patterns than healthy controls, and patients with greater disease severity showed higher overall cortisol values. Furthermore, fatigued breast cancer survivors have shown significantly flatter cortisol slopes compared to their non-fatigued counterparts (4). Polk et al. (22) have reported higher, flatter diurnal cortisol patterns in men with low self-reported positive affect, and Adam et al. (3) reported that greater hours of maternal employment and a greater number of children in the household were associated with lower morning cortisol values and less steep declines across the day. Other work (2) has also shown links between depressive symptoms and lower basal cortisol levels as well as positive relationships between trait anger and the cortisol response to awakening in a community sample of adolescents. Moreover, in an examination of socioeconomic differences in health outcomes, Ranjit and others (24) showed that women with less material hardship experienced both a sharper morning surge and a sharper decline in salivary cortisol in comparison to women with high hardship levels. Our results extend this body of work by showing that greater trait anxiety is associated with the same flattening of the diurnal cortisol rhythm, even in high functioning, healthy, relatively stress resilient individuals.

Our observation of no significant differences between high- and low-anxiety groups on overall cortisol values differs from previous work (22,32). For example, van Eck and colleagues (32) studied the relationship between perceived stress, trait anxiety, depression, and diurnal cortisol variation in a sample of white-collar workers, and demonstrated positive associations of both anxiety and depression with overall cortisol values, but no relationship between overall cortisol values and perceived stress. In addition, Polk et al. (22) measured affect in a large sample of healthy adults across a 3-wk period and showed that trait negative affect was associated with higher total cortisol concentrations and a greater morning rise in men. The different populations of study and/or the different statistical methods used in each study may explain these discrepant findings. In sum, the present findings are continuous with a growing body of research demonstrating higher, flatter diurnal cortisol pat-

terns in association with maladaptive psychosocial functioning and suboptimal environmental circumstances. However, the relationship between psychosocial function and total cortisol levels is less clear. It is plausible, though, that the diurnal pattern is the most important prognostic characteristic, given its established link to morbidity as well as survival rates in clinical populations.

Although high and low trait anxiety groups both exhibited dramatic cortisol responses to SERE, no between-group differences emerged. Few studies have prospectively examined links between psychosocial variables and physiological stress responses to military training. In one exception, Morgan and colleagues (19) observed no relationship of measures of novelty seeking, harm avoidance, or reward dependence to hormonal responses during SERE training. Our current findings are consistent with this previous research in that psychological dispositions were not predictive of the physiological stress response during training. A logical inference of this is that personality variables may have little to do with the physiological response to the stress of intense military training. In that we have shown that high- and low-anxiety individuals differed during daily living but not in response to military stress, a related possibility is that the intensity of SERE training induces a ceiling effect with respect to hormonal responses. The substantial interindividual variability observed in hormonal responses, however, may provide more support for the former. By contrast, although not specifically related to hormonal stress responses, Eid et al. (9) demonstrated that a subscale of a measure of hardiness (i.e., the tendency to view life stressors as a challenge) was negatively associated with peritraumatic dissociation in response to a mild stressor as well as a stressful SERE captivity-related exercise. Morgan et al. (15) also showed that baseline levels of dissociation as well as history of traumatic stress predicted cognitive impairment during SERE training, and, similarly, that dissociative symptoms before and after acute military stress were higher in individuals who reported a perceived threat to life in the past (16). More research is needed to explore possible moderating effects of other psychosocial characteristics (e.g., sensation seeking, extraversion, coping styles) with respect to physiological stress responses as well as key human performance endpoints.

Limitations and Future Research Directions

Limitations of this study should be noted. One limitation concerns the fact that subjects self-administered the salivary samples during the free-living component of the study and were free of direct oversight from the research team (5), which has obvious ramifications should a participant break the ground rules of participation. For instance, although substance addiction was an exclusion criterion and all participants signed a statement of compliance indicating that they had not used any substances or medications 12 h prior to or during the data collection period, acute intake of caffeine, alcohol, and nicotine

cause stress-like cortisol responses and persistent use of these substances may dysregulate the HPA functioning (11,13). To address this, participants were given clear and concise directions regarding administration, and the importance of punctuality was repeatedly expressed by the research staff. A second limitation is the inherent lack of experimental control exerted during a free-living environment. Substantial between-subject variability in daily activities may be possible. Similarly, acute stressors during individual participants' daily lives could influence any given data point across the day. These limitations are moderated to a certain extent, in that all participants were students in military training programs, kept similar standardized military work schedules, and tended to engage in similar daily activities. We also attempted to control for these limitations by averaging values across two consecutive days rather than relying on a single data point for each time period. These limitations are further balanced by the fact that data collection in a free-living environment substantially improves the ecological validity of the study. It should also be noted that the "high-anxiety group" in the current study, although significantly higher than the low-anxiety group, did not score extremely high on the trait anxiety scale. Specifically, the mean score for the high anxiety group is similar to the median score found from a normative college age population (28). That said, the mean trait anxiety scores for the high- and low-anxiety groups in the current study are nearly identical to those of high- and low-anxiety groups in previous research that has successfully demonstrated significant relationships between trait anxiety and cortisol (32). If the high-anxiety group in the present study had demonstrated higher trait anxiety scores, it is reasonable to propose that it would have magnified the observed group differences.

More research is needed to better understand not only the relationships of psychosocial and environmental variables to neuroendocrine function, but also the subsequent link to acute stress reactivity, morbidity, and mortality. Diurnal cortisol profiles, then, may have useful prognostic power, and more work is needed to refine our understanding of their role and that of other hormones and neurotransmitters of the stress system. Another psychosocial variable with great promise for differentiating diurnal cortisol patterns is coping styles. Since coping styles are potentially modifiable, an important and informative line of research may be found in linking adaptive and maladaptive coping styles to diurnal patterns. Subsequently, intervention studies may be performed to determine if behavioral modification can impact the daily patterns, and, as a result, moderate risk factors and improve survival.

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14. ABSTRACT (maximum 200 words) Introduction. Accumulating evidence suggests that negative affect is associated with elevated cortisol. Limited research has investigated this association in young, highly functioning, and stress-resilient populations. Methods. We examined the relation of trait anxiety with total and diurnal salivary cortisol in 26 military men aged 19-30 years (M = 21.6, SD = 2.3). Salivary cortisol was assessed over two consecutive days of free-living measurement at 0730, 0830, 0900, 1630, and 1930. Trait anxiety was measured with the trait portion of the Spielberger State-Trait Anxiety Inventory. Results. Total cortisol concentrations were similar between men reporting high or low anxiety (8.6 ± 3.2 vs. 7.4 ± 2.8 nmol/L, respectively; $p > 0.05$). However, the diurnal cortisol profile differed significantly ($p = 0.04$) between these men. Specifically, men with low anxiety exhibited a diurnal cortisol pattern that peaked in the early morning, decreased precipitously during the midmorning, and continued to decrease throughout the day, reaching a nadir in the evening. By contrast, the cortisol pattern of high-anxiety men remained elevated and significantly higher ($p < 0.05$) than their low-anxiety counterparts during the midmorning, decreased throughout the day, and reached its lowest level in the evening. Results were not substantially altered following adjustment for sleep duration or wake time. Conclusion. These results suggest that the diurnal cortisol pattern in young, apparently healthy men may be influenced by anxiety, although the correlational nature of this study precludes implication of causation. Implications for health and human performance are discussed.					
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